AD_____

Award Number: MIPR 2ECJCN2081

TITLE: Analysis of PSA-Specific T-Cell Responses of Prostate Cancer Patients Given a PSA-Based Vaccine on a Clinical Trial

PRINCIPAL INVESTIGATOR: James Gulley, M.D., Ph.D.
William Dahut, M.D.
Philip M. Arlen, M.D.
Kwong Tsang, Ph.D.
Jeffrey Schlom, Ph.D.

CONTRACTING ORGANIZATION: National Cancer Institute
Bethesda, Maryland 20892

REPORT DATE: April 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2003	Annual Summary (1 Apr 02 - 31 Mar 03)

4. TITLE AND SUBTITLE
Analysis of PSA-Specific T-Cell Responses of Prostate Cancer Patients Given a PSA-Based Vaccine on a Clinical

5. FUNDING NUMBERS
MIPR 2ECJCN2081

6. AUTHOR(S):
James Gulley, M.D., Ph.D., William Dahut, M.D., Philip M. Arlen, M.D., Kwong Tsang, Ph.D., Jeffrey Schlom, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
National Cancer Institute
Bethesda, Maryland 20892

E-Mail: gulleyj@mail.nih.gov

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words) (abstract should contain no proprietary or confidential information)*

Cancer vaccines may improve outcomes by inducing an immune response to tumor antigens. This randomized, phase II clinical trial was designed to determine if a PSA-based vaccine could induce a specific immune response when combined with radiotherapy in patients with localized prostate cancer. The primary endpoint is to identify any immunological response to PSA by monitoring T-cell frequencies using the ELISPOT assay. 29 patients have been randomized into vaccine or no vaccine arms - both receive standard radiotherapy. The vaccine patients receive recombinant vaccinia PSA and B7.1 followed by monthly boosters with fowlpox PSA, as well as GM-CSF and IL-2. No unexpected or severe toxicities have been seen. 11 patients in the vaccine arm have been tested via ELISPOT. 6 showed at least a 3-fold increase in PSA-specific T-cells. None of the 6 tested in the no vaccine arm had an increase. The number of circulating PSA-specific T-cells temporarily decreased following radiotherapy, then returned within 2 months. This may indicate specific cellular trafficking to the prostate. Overall, the PSA-vaccine appears to be well-tolerated and able to produce substantial immune responses, as determined by the ELISPOT assay. It can be safely and, in terms of immune response, effectively combined with local radiotherapy.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
ELISPOT, vaccine, prostate cancer, immunotherapy	11
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction:

Adenocarcinoma of the prostate is the most common cancer diagnosis in American males and follows lung cancer as the leading cause of cancer death (1). Patient survival is related to the extent of tumor. Attention has accordingly focused on definitive treatment strategies for localized disease – surgery and radiotherapy – with generally equivalent results. However, these treatments will fail in 30-40% of patients (2). This trial will seek to pair definitive radiotherapy with an experimental prostate specific antigen (PSA)-based vaccine designed to facilitate an immune response against the prostate cancer. The primary endpoint is to identify any immunologic response to PSA as measured by *in vitro* analysis of the patients' peripheral blood cells using the ELISPOT assay. Cytotoxic CD8+ T lymphocytes (CTL) are important effector cells in immunity against tumor cells, and reliable quantitation of these T-cell frequencies is crucial for the evaluation of specific cancer vaccines (3). The ELISPOT assay has greater sensitivity than the standard CTL assay, specificities of 80-95%, good reproducibility, and is less labor-intensive than the limiting dilution technique (4, 5, 6). Therefore, it was selected to achieve the primary immunologic endpoint of this study.

Methods and Results:

This randomized, phase II clinical trial was initiated to determine if a PSA-based vaccine could induce a specific immune response when combined with radiotherapy in patients with localized prostate cancer. Twenty-nine patients with localized prostate cancer who are HLA-A2 positive have been randomized in a 2:1 ratio into vaccine (cohort A) or no vaccine (cohort B) arms with all patients receiving standard radiotherapy. Those patients in the vaccine arm receive an initial "priming" vaccine with recombinant vaccinia (rV) PSA and rV-B7.1 followed monthly by a booster vaccine with recombinant fowlpox PSA. The vaccines are given with local GM-CSF and low dose systemic IL-2 to enhance the immune response. Radiotherapy commenced after the fourth vaccination. The original accrual ceiling of 30 patients has been increased by 12 patients to accommodate a third cohort, cohort C, which differs from the original vaccine arm only in the dosing schedule of IL-2. The dose has been decreased and is given over 14 rather than 5 days in an attempt to minimize toxicity while optimizing immune response. For all cohorts, the ELISPOT assay is used to monitor T-cell responses to PSA. Each sample is run with an influenza peptide control and samples from a "normal" control HLA-A2+ individual with previously determined levels of influenza-specific T-cell precursors. In addition, each sample is performed with six replicates to control for variability.

Twelve of 18 patients in cohort A have completed all 8 vaccinations. No unexpected or severe toxicities have emerged. The grade 3 toxicities that were seen (primarily fatigue, lymphopenia, and hyperglycemia) were temporally associated with IL-2 +/- radiotherapy. The number of PSA-specific T-cells was quantitated via ELISPOT assay. Six of 11 patients tested had at least a 3-fold increase in PSA-specific T-cell responses over the course of 8 vaccinations, while none of the 6 tested in the no vaccine arm had an increase (Appendix III, IV). Interestingly, the number of circulating PSA-specific T-cells, but not influenza-specific T-cells (control – Appendix V), temporarily decreased immediately following radiotherapy, then returned within 2 months. This may indicate specific cellular trafficking to the prostate. The number of CD4/CD25/CD45RO+ suppressor cells was also determined serially in 6 patients. The only patient who had elevated levels of suppressor cells prior to vaccination had normalization after 3 vaccinations. This decrease in suppressor cells was associated with an increase in PSA-specific T-cells.

Key Research Accomplishments:

Task 1: Analysis of PSA-specific T cells from patients (months 1-18)

a. PSA-specific T-cells will be quantitated from samples taken before vaccination and after all vaccinations via the ELISPOT assay. The comparison of these values will be the primary endpoint of the trial.

This has been completed for 11 of 12 patients who have completed all 8 vaccinations to date. The results of these assays are summarized above and in the American Association of Cancer Research abstract (Appendix I), and detailed in ELISPOT Results, Vaccine Arm (Appendix III).

b. PSA-specific T-cells will also be quantitated from samples taken before and after radiation in both groups of patients.

This has been completed for 11 of 12 patients who have completed all 8 vaccinations to date. In addition, this has been completed in 6 of 7 patients who have completed radiotherapy in the no vaccine arm. The results of these assays are summarized above and in the American Association of Cancer Research abstract (Appendix I), and detailed in ELISPOT Results, Vaccine and Irradiation Only Arms (Appendix III, IV).

Reportable Outcomes:

Abstracts:

1. **James L. Gulley**, William Dahut, Philip M. Arlen, Anne Bastian, Steven Rucker, Jennifer Marte, Patricia Beetham, Dennis Panicali, Claudia Palena, Mahesh Seetharam, Kwong Tsang, Norman Coleman, Jeffrey Schlom. A PSA-based vaccine in a randomized phase II study of patients with localized prostate cancer receiving standard radiotherapy. *Proc. Am. Assoc. Cancer Res.* April, 2003. A LB214 (Appendix 1)

2. **Gulley J**, Dahut W, Arlen PM, Bastian A, Coleman N, Tsang K, Douglas R, Sullivan F, and Schlom J. A PSA-based vaccine in a randomized phase II study of patients with localized prostate cancer receiving standard radiotherapy. *Proceedings Am Soc Clin Onc* 2002; A1814. (Appendix 2)

Conclusions:

The PSA-based vaccine appears to be well-tolerated and able to overcome tolerance to cause substantial specific immunologic responses, as determined by the ELISPOT assay. Assay responses were reproducible, and were not seen with radiation alone despite the potential for local inflammation. The vaccine can be safely and, in terms of immune response, effectively combined with local radiotherapy. It is too early to determine any clinical results.

References:

1. Coffey DS. Prostate Cancer: an overview of an increasing dilemma. *Cancer* 71 (Suppl 1):880-886, 1993.

2. American Urological Association Prostate Cancer Clinical Guidelines Panel. Report on the management of clinically localized prostate cancer. Baltimore: American Urological Association, 1995.

3. Boon T, Gajewski TF, and Coulie P. From defined tumor antigens to effective immunization? *Immunology Today* 16:334 Abstract, 1995.

4. Miyahira Y, Murata K, Rodriguez JR, Esteban M, Rodrigues MM, and Zavala F. Quantification of antigen specific CD 8+ T cells using an ELISPOT assay. *J Imm Meth* 1995 181(1):45-54.

5. Scheibenbogen C, Lee K, Mayer S, Stevanovic S, Moebius U, Herr W, Rammensee H, and Keilholz U. A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specific for HLA class I-binding peptide epitopes derived from influenza proteins in the blood of healthy donors and melanoma patients. *Clin Cancer Res* 1997 3:221-226.

6. Schmittel A, Keilholz U, and Scheibenbogen C. Evaluation of the interferon-gamma ELISPOT-assay for quantification of peptide specific T lymphocytes from peripheral blood. *J Imm Meth* 1997 210:167-174.

Appendix I – American Association of Cancer Research Abstract

A PSA-based vaccine in a randomized phase II study of patients with localized prostate cancer (PC) receiving standard radiotherapy

James Gulley[1], William Dahut[2], Philip Arlen[1], Anne Bastian[2], Steven Rucker[1], Jennifer Marte[1], Patricia Beetham[1], Claudia Palena[1], Mahesh Seetharam[1], Kwong Tsang[1], Norman Coleman[3], Dennis Panicali[4], Jeffrey Schlom[1], [1]Laboratory of Tumor Immunology and Biology, CCR, [2]Medical Oncology Clinical Research Unit, CCR, [3]Radiation Oncology Branch, CCR, NCI, Bethesda, MD, [4]Therion Biologics Corporation, Cambridge, MA

Introduction: We have shown that PSA-encoding poxvirus vaccines are safe and can induce increases in PSA-specific T cells in metastatic PC; however, advanced cancer may not be an optimal setting for immunotherapy. Treatment of localized PC includes surgery and radiotherapy; however, these treatments will fail in 30-40% of patients. Vaccine therapy has not been implemented in the localized disease setting, yet may be clinically more effective in patients with lower systemic tumor burdens. Recent work in our lab has shown that costimulatory molecules, cytokines and radiation further potentiate the immune response. **Methods:** We present a novel randomized phase II clinical trial designed to determine if a PSA-based vaccine can induce a specific immune response when combined with radiotherapy in patients with localized PC. Twenty-eight patients with localized PC who are HLA-A2 positive were randomized in a 2:1 ratio into vaccine or no vaccine arms with all patients receiving standard radiotherapy. Those patients in the vaccine arm received an initial "priming" vaccine with recombinant vaccinia (rV) PSA and rV-B7.1 followed monthly by a booster vaccine with recombinant fowlpox PSA. The vaccines were given with local GM-CSF and low dose systemic IL-2 (a week after vaccine). Radiotherapy commenced after the 4th vaccination. The ELISPOT assay was used to monitor T-cell responses to PSA. **Results:** Eleven of 16 patients in the vaccine arm have completed all 8 vaccinations. No unexpected or severe toxicities have emerged. There have been 109 vaccine cycles given with a total of 11 cycles with grade 3 toxicity (fatigue, fever, hyperglycemia), all temporally related to the IL-2. The number of PSA-specific T-cells was quantitated via ELISPOT assay. Six of 11 vaccine patients tested had at least a 3-fold increase in PSA-specific T-cell responses over the course of 8 vaccinations, while none of 6 in the no vaccine arm had an increase. The number of CD4/CD25/CD45RO+ suppressor cells was also determined serially in 6 patients. The only patient who had elevated levels of suppressor cells prior to vaccination had normalization after 3 vaccinations. This decrease in suppressor cells was associated with an increase in PSA-specific T-cells. Interestingly, the number of circulating PSA-specific T-cells, but not influenza-specific T-cells (control), temporarily decreased immediately following radiotherapy, then returned within 2 months. This may indicate specific cellular trafficking to the prostate. It is too early to determine any clinical results. **Conclusions:** This appears to be a well-tolerated vaccine that is able to overcome tolerance to cause substantial specific immunologic responses. These responses were not seen with radiation alone despite the potential for local inflammation. The vaccine can be safely and, in terms of immune response, effectively combined with local radiotherapy.
Funding: DOD Prostate Cancer Research Program DAMD17-02-IA-0004

Appendix II – American Society of Clinical Oncology Abstract

A Prostate Specific Antigen (PSA)-based vaccine in patients (pts) with localized prostate cancer (pc) receiving standard radiotherapy (RT)

James L. Gulley, William Dahut, Philip Arlen, Anne Bastian, Norman Coleman, Kwong Tsang, National Cancer Institute, Bethesda, MD, Robert Douglas, National Naval Medical Center, Bethesda, MD Frank Sullivan, Silver Spring Cancer Care, Silver Springs, MD, Jeffrey Schlom, National Cancer Institute, Bethesda, MD,

Background: Typical treatment failure rates for localized pc range from 30-50% depending on pretreatment risk factors. Adjunct therapies such as vaccines given with definitive therapy may improve outcomes. Methods: A randomized phase II clinical trial was initiated to determine if a PSA-based vaccine could induce a specific immune response when combined with radiotherapy in pts with localized pc. Pts were randomized in a 2:1 ratio to vaccine or no vaccine arms. They received an initial priming with recombinant vaccinia (rV)-PSA and rV-B7.1 followed by monthly boosts with rF-PSA for a total of 8 vaccinations before, during and after RT. Vaccines were given with local GM-CSF (100 mcg) and low dose systemic IL-2 (4 mu/m2). Pts were required to get external beam RT +/- brachytherapy, +/- hormonal therapy. Results: Fourteen of 30 pts have been enrolled (9 vaccine, 5 no vaccine) with median on study PSA 6.13 (range 0.17-122). Seven pts have completed the trial (4 on vaccine arm). Of 52 cycles of vaccine, there were 2 pts with grade 3 hyperglycemia (both known diabetics) and 3 pts with grade 3 fatigue (2 during radiation therapy). There were no other grade 3 or 4 toxicities. Five pts had dose reductions in IL-2 for the above toxicities. All grade 3 toxicities were at least partially attributed to IL-2. There were no apparent increases in RT toxicity. Conclusions: This vaccine regimen appears to be well tolerated in pts with localized pc. Results of quantitation of PSA-specific T cells at various time points (pre vaccine, before RT, immediately after RT and 1 month after all vaccinations with similar time points in the no vaccine arm) via ELISPOT assay are forthcoming and pts will be followed for 2 years with additional immune and clinical monitoring.

Appendix III - ELISPOT Results, Vaccine Arm

Vaccination Arm	Patient	Sample	Flu Peptide	PSA3 Peptide
1	RM3178	pre	1/23,077	<1/200,000
		post 3	1/21,429	<1/200,000
		post 5	1/25,000	<1/200,000
		post 8	1/21,429	<1/200,000
2	DG7283	pre	1/14,286	<1/200,000
		post 3	1/8,955	1/26,087
		post 5	1/14,286	<1/200,000
		post 8	1/13,363	1/50,000
3	DC9243	pre	1/100,000	<1/200,000
		post 3	1/150,000	1/50,000
		post 5	1/100,000	<1/200,000
		post 8	1/150,000	1/46,154
4	LN3447	pre	1/42,857	1/50,000
		post 3	1/46,154	1/37,500
		post 5	1/42,857	1/24,000
		post 8	1/46,154	1/15,789
5	LM9618	pre	1/31,579	1/50,000
		post 3	1/20,690	1/46,154
		post 5	1/46,154	<1/200,000
		post 8	1/13,953	1/17,143
6	BF5181	pre	1/21,429	1/85,714
		post 3	1/20,000	1/54,545
		post 5	1/21,429	1/66,667
		post 8	1/13,043	1/22,222
7	PD4701	pre	1/18,182	<1/200,000
		post 3	1/27,273	1/42,857
		post 5	1/35,294	1/26,087
		post 8	1/11,765	1/15,000
8	PB4229	pre	1/26,087	1/85,714
		post 3	1/11,111	1/54,545
		post 5	1/16,216	1/150,000
		post 8	1/15,385	1/100,000
9	DP5500	pre	1/75,000	1/100,000
		post 3	1/60,000	1/85,714
		post 5	1/54,545	1/120,000
		post 7	1/75,000	<1/200,000
10	AM3587	pre	1/46,154	1/100,000
		post 3	1/37,500	1/150,000
		post 5	1/54,545	<1/200,000
		post 8	1/35,294	1/200,000
11	NS2834	pre	1/60,000	1/150,000
		post 3	1/60,000	1/37,500
		post 5	1/54,545	1/150,000
		post 8	1/60,000	1/200,000

Appendix IV – ELISPOT Results, Irradiation Only Arm

Irradiation Only Arm	Patient	Sample	Flu Peptide	PSA3 Peptide
1	DH1606	pre	1/66,667	<1/200,000
		Pxt	1/60,000	<1/200,000
		Pxt+3	1/54,545	<1/200,000
2	WW0452	pre	1/46,154	<1/200,000
		Pxt	1/40,000	<1/200,000
		Pxt+3	1/30,000	<1/200,000
3	AP7087	pre	1/60,000	<1/200,000
		Pxt	1/54,545	<1/200,000
		Pxt+3	1/66,667	<1/200,000
4	LT3150	pre	1/12,766	<1/200,000
		Pxt	1/10,204	<1/200,000
		Pxt+3	1/12,000	<1/200,000
5	JU7147	pre	1/40,000	<1/200,000
		Pxt	1/37,500	<1/200,000
		Pxt+3	1/40,000	<1/200,000
6	JC8715	pre	1/13,333	<1/200,000
		Pxt	1/11,364	<1/200,000
		Pxt+3	1/14,286	<1/200,000

Pxt = post irradiation, time equivalent to post 5 vaccination
Pxt+3 = post irradiation plus 3 weeks, time equivalent to post 8 vaccination

Appendix V – ELISPOT Results, Normal Donor Control

Date	Flu Peptide
7/29/02	1/14,634
7/30/02	1/14,286
8/19/02	1/20,000
8/20/02	1/14,286
8/26/02	1/16,216
8/27/02	1/17,143
9/2/02	1/13,636 1/13,954
9/9/02	1/16,667
9/17/02	1/13,636 1/13,333
9/30/02	1/18,750 1/19,355 1/20,000
10/15/02	1/15,385 1/13,043 1/12,500
12/3/02	1/12,500 1/12,766

PSA3 and PSA3a peptide precursor frequencies were always <1/200,000